Editorial

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HIF-1α is a major and complex player in alcohol induced liver diseases

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Hepatic steatosis is one of the earliest and most consistent changes which occur with excessive consumption of alcohol. The development of steatosis is known to sensitize the liver to other insults, and is an important step towards the development of the full spectrum of alcohol-induced liver pathologies [1]. The combination of high energy consumption by hepatocytes, and the venous inflow into the liver place hepatocytes at risk of hypoxia. Alcohol consumption was shown to increase hepatic oxygen consumption, with a smaller increase in hepatic oxygen delivery, resulting in central venous hypoxia [2]. These findings bring together the fields of alcoholic liver disease and tissue adaptation to hypoxia. Adaptation to low oxygen tension is known to be regulated by transcriptional induction of genes important in myriad biological processes including metabolism, angiogenesis, cell cycle regulation, and cell death. The hypoxia inducible factor (HIF) family of heterodimeric transcription factors is important in regulating these processes [3]. Functional HIF is composed of an alpha unit (HIF-1α, HIF-1β, or HIF-3α), and a HIF-1β subunit. Under normal conditions, the alpha units are rapidly degraded in the cytosol, but under hypoxic conditions they escape degradation, dimerise with the β unit, translocate to the nucleus, and regulate numerous gene transcription programs [4].

In this issue of the *Journal*, Dr. Nishiyama and colleagues address the question of the role of HIF-1α in the metabolic and histological changes associated with alcoholic liver disease [5]. They employ a sophisticated but well established technique of hepatocyte specific HIF-1α deletion, and demonstrate that, in a model of alcohol-induced liver disease in mice, there is an up-regulation of HIF-1α in a central venous distribution, and, in the absence of hepatocyte HIF-1α, there is greater pathology with increased liver weight, steatosis and increased liver and serum triglycerides. This is strong evidence that the alcohol-induced increase in hepatic HIF-1α is adaptive and protective. To understand which genes are regulated by HIF-1α in this alcohol-induced liver disease model, the authors identified changes in expression levels of genes known to be important in lipid metabolism, and found higher expression levels for a number of lipogenic and fatty acid oxidation genes. Due to the kinetics of sustained elevation, they focused on two genes coding for the lipogenic proteins SREBP-1c, and its downstream target ACC. A HIF-1α-induced transcriptional regulator repressor DECI was demonstrated to be important for maintaining a lower level of SREBP-1c in wild-type mice.

A recent study also found that an alcohol rich diet up-regulates HIF-1α in mice, but subsequently demonstrated that HIF-1α, rather than inducing adaptive and protective changes from the development of steatosis, is a key step in the development of steatosis and hypertriglyceridemia [6]. This study used two genetic manipulations, one in which there was constitutively active HIF-1α in hepatocytes, and a second that used the same hepatocyte specific HIF-1α deletion approach as above. Both genetic manipulations gave concordant results, with mice expressing constitutively active HIF-1α in hepatocytes developing increased liver steatosis and hypertriglyceridemia, and mice lacking HIF-1α in hepatocytes having reduced liver steatosis and hypertriglyceridemia.

Both studies used similar and at times near identical technologies, and the reasons for the opposite findings are not obvious. Focusing on potential weak points, over-expression studies are at risk of not capturing the scale, timing and tissue distribution of native expression. This is certainly possible for HIF-1α which has a zonal distribution, and expression peaks one hour after hypoxia [5,7]. The discordant results between the two studies when using the same mice lacking HIF-1α in hepatocytes are much more difficult to explain. There are differences in the diets used, and also in the choice of controls which appear minor. Nishiyama *et al.* used littermate HIF-1α floxed mice without Alb-Cre transgene as controls, and Nath *et al.* used wild-type mice. An analogous discrepancy is present in the literature for c-Jun N terminal kinase 2 (JNK2) deleted mice with acetaminophen (APAP) toxicity. One group demonstrated that *JNK2*−/− mice had greater susceptibility to APAP toxicity, and another group demonstrated *JNK2*−/− mice had reduced susceptibility [8,9]. This was resolved by the careful demonstration that differences in mouse substrains, which were not recognized by the two groups and by the vendor, made the crucial difference [9]. Finally, there are likely different colonic flora in in-house and purchased mice, which may result in different responses to alcohol feeding and

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gut endotoxin permeability, such that there is a greater TLR4 signal along with ethanol in one colony compared to another. Differences in colic flora have recently been shown to have a major effect on colitis phenotypes [10]. There is no reason to believe that any of these factors are relevant here or in many other studies, however, when faced with such different results, future studies on HIF-1α would hopefully remove such potentially confounding factors.

A different approach to address these data is to compare which model fits best with the other published data on HIF-1α and liver pathology. For other pathological responses is HIF-1α responsible for adaptive changes which counter a pathological insult, or is it a key step which converts the signal from the pathologic stressor to a full pathologic phenotype? Staying with models of alcohol induced injury, if we examine the inflammatory responses, the role of HIF-1α has been studied for up-regulation of RANTES/CCL5, which is a member of the superfamily of proinflammatory cytokines referred to as the CC chemokine family, and it is chemoattractant for T lymphocytes, monocytes, and macrophages. The upregulation of RANTES/CCL5 is in part due to HIF-1α, suggesting a direct role for HIF-1α in the alcohol induced inflammatory response [11]. Ethanol also induces increases in portal pressure which is dependent on upregulation of endothelin-1, which is mediated by HIF-1α. This suggests that HIF-1α is a mediator towards the development of a pro-inflammatory and vasoconstrictive phenotype in alcoholic liver disease [12].

Chronic hypoxia is also known to occur in many models of liver fibrosis, and as expected, HIF-1α is activated in fibrotic livers. In a model of bile duct ligation induced fibrosis, inducible deletion of HIF-1α resulted in lower levels of several pro-fibrogenic mediators, less α-smooth muscle actin, less hepatic collagen and reduced liver fibrosis [13]. Liver fibrosis is also associated with angiogenesis, and analogous to a role in liver fibrosis, in the absence of HIF-1α there was a significant reduction in up-regulation of the vascular endothelial growth factor.

In hepatic inflammation, portal hypertension, liver fibrosis, and angiogenesis, HIF-1α appears to be a mediator of the disease phenotype, rather than a regulator against it. In relation to steatosis, a recent study suggests that the situation may be more complex than a simple binary analysis of whether HIF-1α is driving pathological phenotype, or protecting against it. A factor inhibiting HIF-1α (FIH) blocks the association of HIFs with transcriptional co-activators, thus inhibiting HIF mediated transcriptional activation. FIH null mice have increased HIF functionality, and a large range of metabolic changes including a higher metabolic rate, hyperventilation, improved glucose control and resistance to high-fat-diet-induced steatosis [14]. The question of whether these changes in FIH null mice are due to increased HIF mediated transcription, or to additional pathways still needs to be addressed.

Collectively, these studies demonstrate hepatic HIF-1α is increased in mice after an alcohol rich diet and manipulation of HIF-1α *in vivo* has profound effects on the steatotic and metabolic phenotypes after high alcohol consumption. As HIF-1α is a transcription regulator rather than a receptor ligand or enzyme, it is not surprising that the results of changing HIF-1α activity are complex, and may be very dependent on which other signals the hepatocyte is receiving at the same time. Unraveling these complexities will be very challenging, and may require approaches analogous to other complex problems such as study of drug induced toxicity. Significant progress has been made with novel approaches such a network of investigators rather than individual laboratories [15].

Conflict of interest

The author declared that he does not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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